



# A 2010 Austrian *Salmonella enteritidis* PT4 outbreak associated with a laying hen holding previously involved in an *S. enteritidis* PT4 cluster: Pitfalls of regulatory responses in risk management

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## KEYWORDS

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**Summary** We report on an outbreak caused by *Salmonella enteritidis* phage type 4 (PT4) among 143 participants at a soccer camp in Austria in August 2010. The outbreak affected 34 persons, including 24 epidemiologically related cases and 10 laboratory-confirmed cases. Food-specific cohort analyses revealed spaetzle (homemade noodles) (relative risks (RR): 2.68; 95% CI: 1.13–6.45), hamburger (RR: 2.70; 95% CI: 1.13–6.45) and potato salad (RR: 2.91; 95% CI: 1.69–5.02) as the most biologically plausible infection sources. Eggs used as ingredients were considered to be the vehicle of infection for the outbreak strain. The sole egg producer supplying the hotel that housed the soccer camp participants with table eggs operated two flocks. One flock had been epidemiologically and microbiologically related to a previous *S. enteritidis* PT4 outbreak affecting the same Austrian province in the four months preceding the August outbreak. We hypothesize that eggs from this flock, already condemned for industrial use only, were falsely declared table eggs and sold among eggs from the non-banned flock causing the subsequent outbreak. In Austria, the illegal distribution of eggs designated for industrial use (i.e., false declaration of these eggs as table eggs) has been

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previously documented. Our findings underscore the potential of proper epidemiological outbreak investigation to identify the pitfalls of regulatory responses in risk management.

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## Introduction

Non-typhoidal *Salmonella* is a foodborne zoonotic pathogen with great potential for causing foodborne outbreaks [1]. In Austria, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*Salmonella enteritidis*) is the most frequent serotype, causing 77.6% of human salmonellosis cases ( $N = 28,482$ ) from 2004 to 2009 [2]. Eggs and egg products are the most frequent sources of infection for *S. enteritidis* [1]. The most relevant reservoirs of *S. enteritidis* are laying hen flocks [3], and the vaccination of laying hens against *S. enteritidis* has been mandated in Austria since 2008 [4]. We report the findings on an outbreak of gastroenteritis caused by *S. enteritidis* phage type (PT) 4 among participants of a soccer camp in Austria in August 2010.

## Outbreak description

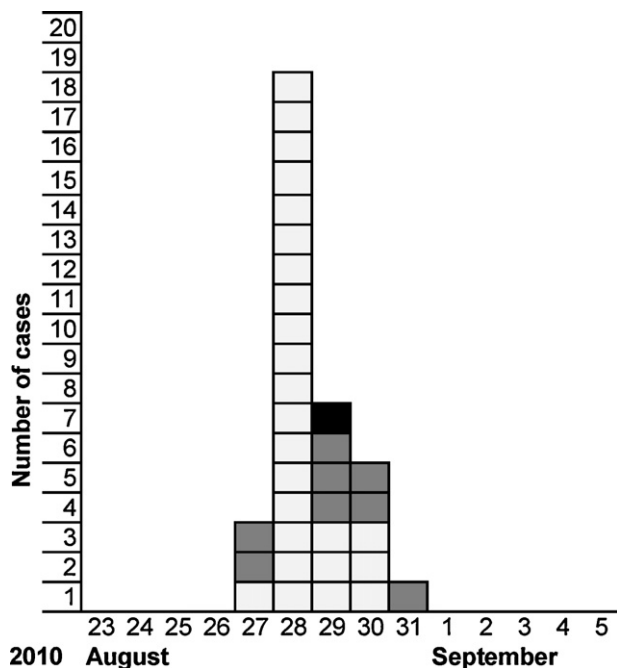
At the end of August 2010, ten cases of gastroenteritis occurred among the participants of a soccer camp that was held in the eastern Austrian province of Burgenland the week of August 21–27. Stool samples were available for five cases, and all tested positive for *S. enteritidis* PT4. On September 15, the Austrian Agency for Health and Food Safety (AGES) was mandated to investigate the suspected foodborne outbreak among the soccer camp participants. A total of 143 persons from five soccer clubs originating from three neighboring provinces in eastern Austria attended the soccer camp and were accommodated in a hotel (hotel X) from August 21 at the earliest until August 29 at the latest. The staff of hotel X comprised 12 persons, including the kitchen and cleaning staff.

The outbreak case definitions were as follows: a probable outbreak case was defined as a person who (i) participated in the soccer camp from August 21 until August 29 and (ii) fell ill on August 22 at the earliest with symptoms of gastroenteritis. A confirmed outbreak case was defined as a person fulfilling criteria (i) and (ii) who had a laboratory-confirmed infection with *S. enteritidis* PT4. Active case findings were conducted among the participants of the soccer camp.

A total of 34 persons fulfilled the outbreak case definition, including 24 probable and 10 confirmed

outbreak cases among the 143 participants of the soccer camp, yielding an attack rate of 23.8%. The median age of the cases was 12.8 years (min: 9 years; max: 37 years) with a male:female ratio of 31:3. Nausea (31/34), cramps (30/34) and diarrhea (31/34) were the predominant symptoms among the outbreak cases. The outbreak lasted from August 27 until August 31 and peaked with 18 cases (53%) on August 28 (Fig. 1). Considering the usual maximum incubation period of 3 days for salmonellosis, food items served by the hotel X kitchen from August 24 (3 days prior to August 27) until August 28 were tested as potential risk factors for gastroenteritis. By August 29, the kitchen was already closed.

A retrospective cohort study was conducted to identify the food item(s) most likely to be associated with the risk of infection with the outbreak strain *S. enteritidis* PT4 and to generate a hypothesis on the likely disease reservoir.



**Figure 1** Outbreak cases by date of onset ( $N = 34$ ); cases among soccer club A ( $n = 25$ ) illustrated by bright gray square, case ( $n = 1$ ) among soccer club B by black square and cases ( $n = 8$ ) among soccer club D by dark gray square.

## Materials and methods

### Analytical-epidemiological investigation

A list of the soccer camp participants was provided by the trainers. The cohort of interest comprised 143 persons and included 123 players from five soccer clubs plus 20 other persons, including trainers and accompanying persons (club A:  $n=62$ , B:  $n=34$ , C:  $n=17$ , D:  $n=14$  and E:  $n=16$ ). The camp participants originated from three different provinces (Vienna, Lower Austria and Styria).

The food items served from August 24 to August 28, 2010, were defined as exposure factors of interest. Information on these food items was provided by the kitchen manager of hotel X and included a total of 55 different items. All five clubs, A, B, C, D and E, received the same breakfast buffet (including cheese, sausage, milk, hot chocolate, corn flakes, bread, jam, butter and boiled or fried eggs). Identical lunch and dinner dishes were offered to each of the five clubs, but on different days during the camp week. Knowledge of the particular day on which the dishes for lunch and dinner were served was available only for soccer club A (Table 1). The data on the consumption of these food items were ascertained by a self-administered questionnaire, telephone or face-to-face interview. Food-specific attack rates and relative risks (RR) were calculated for the food items regardless of the day on which they were served. In a second approach, food-specific cohort analyses were performed day-wise for each of the days from August 24 until August 28 using soccer club A, which was the largest participating club and the only club with documentation of the dates the dishes were offered. A study cohort was defined for each day (i.e., day-specific study cohorts for August 24–28) for club A participants. Outbreak cases occurring prior to and on the day under study were excluded from the day-specific study cohort. A diseased person was defined as a member of the day-specific study cohort who contracted gastroenteritis within 3 days following exposure to the food item of the specific day under study (considering a maximum incubation period of 72 h). The data were entered into Epi-Info version 3.5.1, and STATA version 11 was used for univariate and stratified analyses. Differences in the food-specific attack rates (AR) between the exposed and unexposed groups were tested by chi-squared test or Fisher's exact test, which yielded relative risks with 95% confidence intervals. A difference was considered to be significant at the 5% level.

### Microbiological investigation

Stool specimens from the 12 staff members of hotel X, none of whom showed symptoms of gastroenteritis, were tested for *Salmonella* spp. Environmental samples obtained from the hotel X kitchen (including 20 Rodac plates from the salad preparation site, trays, refrigerator handle, slicing machine and sink faucet) and from the laying hen holding (including two dust samples of 150 g and five paired boot swabs per flock) that had provided eggs to hotel X during the relevant time period were processed as previously described [5,6]. Stool samples from 10 of the outbreak cases were available for testing for enteric pathogens, including *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter* and *Escherichia coli*. Human and non-human isolates were serotyped according to the Kauffmann–White scheme [7], phage typed [8] and genotyped using variable number of tandem repeats (VNTR)-analysis [9] and pulsed-field gel electrophoresis (PFGE) performed with the restriction enzyme *Xba*I [10].

## Results

### Analytical-epidemiological investigation

Data on the exposure factors under study were available for 126 of 143 persons of the cohort of interest (a response rate of 88%), giving a final cohort of 108 soccer camp participants, 15 trainers and 3 other accompanying persons (soccer club A:  $n=61$ , B:  $n=34$ , C:  $n=17$ , D:  $n=14$  and E:  $n=0$ ).

The crude food-specific cohort analyses with the 55 food items served on August 24–28 yielded consumption of spaetzle (homemade noodles) prepared by boiling small lumps of dough made of flour and eggs, grilled chicken legs, hamburgers made out of minced meat and eggs, pork cutlets in mushroom sauce, mixed vegetables and lasagna as factors significantly associated with infection risk (Table 2a). When applying the food-specific analyses by day using soccer club A as the study cohort, the food items spaetzle, served for dinner on August 25 (RR: 2.41; 95% CI: 0.80–7.30;  $p=0.081$ ), hamburger (RR: 3.57; 95% CI: 1.19–10.73;  $p=0.006$ ) and mashed potatoes (RR: 2.53; 95% CI: 1.07–5.96;  $p=0.018$ ), served together at lunch on August 26, and potato salad (RR: 2.91; 95% CI: 1.69–5.02;  $p=0.003$ ), served at dinner on August 26, were identified as associating with infection risk. Table 2b displays the results of the day-wise food-specific analyses performed with the club A cohort ( $n=61$ ). The results are given for

**Table 1** Dishes by day served to soccer club A.

Day of exposure	Meal	Dishes/food items
24.08.2010	Lunch	Assortment of 4 salads Potato salad Green salad Cabbage salad Corn salad Noodle soup Pork cutlet/mushroom sauce/rice
	Dinner	Assortment of 4 salads as shown above Curd cheese cream Ice cream Sausage salad Pancakes
25.08.2010	Lunch	Assortment of 4 salads as shown above Chicken strips/noodles) Mixed vegetables Soup with croutons
	Dinner	Assortment of 4 salads as shown above Breaded pork cutlet/French fries Goulash with spaetzle Ice cream Cheese and ham toast
26.08.2010	Lunch	Assortment of 4 salads as shown above Soup with semolina dumplings Hamburger/mashed potato Roll with breaded pork cutlet
	Dinner	Soup with semolina dumplings Grilled chicken legs/rice/French fries Assortment of salads as shown above (including potato salad) Ice cream
27.08.2010	Lunch	Assortment of 4 salads as shown above Soup with pancake stripes Breaded cutlet/French fries
	Dinner	Lasagne Cheese and ham toast Assortment of salads as shown above Ice cream/cake
28.08.2010	Lunch	Assortment of 4 salads as shown above Soup Pizza Beef cutlet/rice Ice cream
	Dinner	Grilled sausages/cutlet/chicken legs/grilled potatoes

dinner on August 25 and for lunch and dinner on August 26. The analyses performed with the food items served at lunch and dinner on August 24, 27 and 28, at lunch on August 25 and at breakfast on August 24–28 yielded no risk-associations (data not shown).

After stratifying the risk analysis of the consumption of mashed potatoes by the exposure status to hamburger, eating mashed potatoes was no longer risk-associated. A total of 19 (86%) of the 22 cases

among soccer club A, which had onset of illness between August 27 and 29, consumed hamburger at lunch on August 26, and 8 of these 22 cases (36%) ate potato salad served at dinner on August 26. Spaetzle, hamburger and the potato salad of August 26 were the most biologically plausibly food items associated with infection risk. Eggs used as ingredients for spaetzle and hamburger were considered to be the likely vehicle of the outbreak strain.

**Table 2a** Significant findings of the crude (day-independent), food-specific analyses with the total cohort ( $n = 126$ ); food specific-attack rates (AR%), risk ratio, 95% confidence intervals [95% CI],  $p$ -values.

Food items	Food exposed			Food unexposed			Risk ratio [95% CI]	$p$
	Total	Cases	AR%	Total	Cases	AR%		
Spaetzle	80	28	35.0	46	6	13.0	2.68 [1.20–5.99]	0.008
Grilled chicken legs	77	27	35.1	49	7	14.29	2.45 [1.16–5.20]	0.01
Hamburger	86	29	33.7	40	5	12.5	2.70 [1.13–6.45]	0.012
Roasted pork cutlets	78	27	34.6	48	7	14.6	2.37 [1.12–5.02]	0.014
Mixed vegetables	30	13	43.3	96	21	21.9	1.98 [1.13–3.46]	0.021
Lasagna	69	24	34.8	57	10	17.5	1.98 [1.04–3.79]	0.03

### Microbiological investigation

A laying hen holding comprising two flocks (A and B) in the Austrian province of Burgenland, where the soccer camp was held, was traced as the sole source

providing table eggs for hotel X. On September 14, flock B was examined during the investigation of the soccer camp outbreak by collecting five paired boot swabs and two dust samples. All of the samples from flock B tested negative for *Salmonella* spp.

**Table 2b** Findings of the day-wise, food-specific analyses with the study-cohort *soccer club A* ( $n = 61$ ); results are given for dinner on August 25, and for lunch and dinner on, August 26; food specific-attack rates (AR%), risk ratio, 95% confidence intervals [95% CI],  $p$ -values.

Meal	Food items	Food exposed			Food unexposed			Risk ratio [95% CI]	p
		Total	Cases	AR%	Total	Cases	AR%		
August 25									
Dinner	Spaetzle	42	16	38.1	19	3	15.8	2.4 [0.8–7.3]	0.081
	Green salad	17	8	47.1	44	11	25.0	1.9 [0.9–3.9]	0.095
	Corn salad	3	2	66.7	58	17	29.3	2.3 [0.9–5.6]	0.226*
	Breaded pork cutlet	11	5	45.5	50	14	28.0	1.6 [0.7–3.6]	0.294*
	Potato salad	9	4	44.4	52	15	28.9	1.5 [0.7–3.6]	0.441*
	Cabbage salad	5	2	40.0	56	17	30.4	1.3 [0.4–4.1]	0.643*
	Ice cream	5	1	20.0	56	18	32.1	0.6 [0.1–3.7]	0.643*
	French fries	10	4	40.0	51	15	29.4	1.4 [0.6–3.3]	0.710*
	Gulasch	34	11	32.5	27	8	29.6	1.1 [0.5–2.3]	0.82
Toast	1	0	0.0	60	19	31.7	0.0 [–]	1.000*	
August 26									
Lunch	Hamburger	39	19	48.7	22	3	13.6	3.6 [1.2–10.7]	0.006
	Mashed potatoes	35	17	48.6	26	5	19.2	2.5 [1.1–6.0]	0.018
	Green salad	9	5	55.6	52	17	32.7	1.7 [0.8–3.4]	0.263*
	Roasted pork cutlet	11	2	18.2	49	19	38.8	0.5 [0.1–1.7]	0.299*
	Dumpling soup	11	4	36.4	49	17	34.7	1.1 [0.4–2.5]	1.000*
	Potato salad	3	1	33.3	58	21	36.2	0.9 [0.2–4.7]	1.000*
	Cabbage salad	3	1	33.3	58	21	36.2	0.9 [0.2–4.7]	1.000*
	Corn salad	1	0	0.0	60	22	36.7	0.0 [–]	1.000*
Dinner	Potato salad	10	8	80.0	51	14	27.5	2.9 [1.8–5.0]	0.003*
	Green salad	11	6	54.6	50	16	32.0	1.7 [0.9–3.3]	0.182*
	Grilled chicken legs	43	17	39.5	18	5	27.8	1.4 [0.6–3.3]	0.383
	Ice cream	9	2	22.2	52	20	38.5	0.5 [0.2–2.1]	0.467*
	Dumpling soup	11	3	27.3	49	18	36.7	0.7 [0.3–2.1]	0.731*
	Rice	40	15	37.5	21	7	33.3	1.1 [0.5–2.3]	0.787*
	Cabbage salad	4	1	25.0	57	21	36.8	0.7 [0.1–3.8]	1.000*
	Corn salad	2	1	50.0	59	21	35.6	1.4 [0.3–5.9]	1.000*
	French fries	8	3	37.5	53	19	35.9	1.1 [0.4–2.7]	1.000*

\* Fisher's exact test.



Flock A was not investigated at this time because a marketing ban had already been imposed on flock A on July 14 following the detection of *S. enteritidis* PT4 during the investigation of a previous outbreak of 4 cases with disease onset from May until July 2010 in the same province.

The *S. enteritidis* PT4 isolates recovered from the 10 laboratory-confirmed cases of the soccer camp outbreak were indistinguishable by VNTR-analysis and PFGE from the four human *S. enteritidis* PT4 isolates obtained from the previous flock A-related PT4 outbreak, from the non-human PT4 isolates recovered from the two dust samples and from 4 of 100 pools of 40 eggs that had been sampled from flock A on July 21 (Fig. 2). All of the PT4 isolates shared the VNTR pattern 8-6-5.

The stool specimens from the 12 staff members along with the 20 environmental samples (Rodac plates) obtained from the kitchen of hotel X all tested negative for *Salmonella* spp. Inspection of the kitchen by public health authorities on September 9 revealed a lack of food safety practices in terms of unclean cooking surfaces (including stoves, ovens and grills), unclean surfaces in the storage room and refrigerators, food storage without adequate covering and poor knowledge of appropriate hand hygiene practices among the kitchen staff.

## Discussion

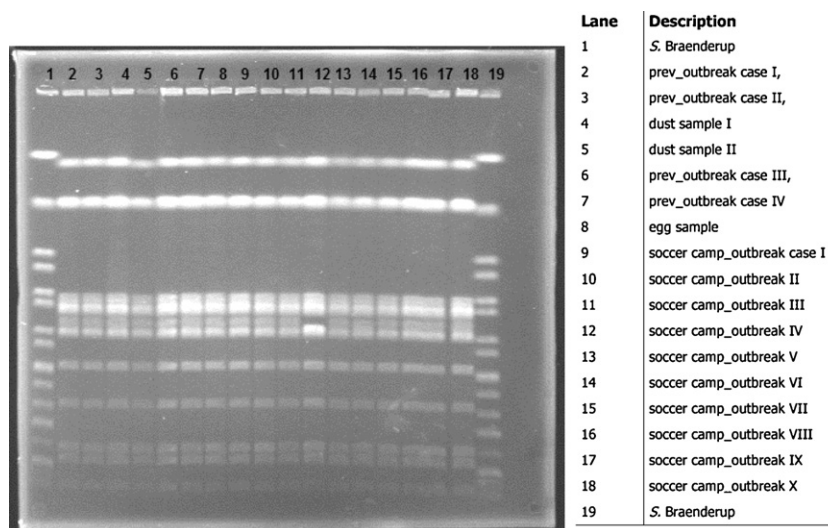
In this analysis, we report on a foodborne outbreak of gastroenteritis due to *S. enteritidis* PT4 among 143 participants of a soccer camp that stayed at a hotel in an eastern province of Austria the last week of August 2010. The food-specific cohort analyses revealed spaetzle (egg-containing homemade noodles), egg-containing hamburger, grilled chicken legs, roasted pork cutlets in mushroom sauce, mixed vegetables and lasagna as being significantly associated with infection risk. According to the testimony of the interviewed cohort-members of this outbreak, the chicken legs should have been well grilled. Chicken meat has recently been shown to be of minor risk for salmonellosis in Austria [11]. Based on the preparation procedures, the lasagna (prepared with commercially produced noodles), stewed mixed vegetables and roasted pork cutlets also appeared to be unlikely sources of infection with the outbreak strain.

The spaetzle and hamburger showed a strong association with infection risk (RRs of 2.68 and 2.70, respectively); the consumption of spaetzle could explain 28 (82%) and hamburger 29 (85%) of the 34 outbreak cases. Both dishes

were prepared with table eggs. In a previously described gastroenteritis outbreak due to *S. enteritidis* PT4 in Austria in 2006, spaetzle was identified by analytical–epidemiological investigation as the most likely source of infection for at least 94% of the total 35 cases [12]. Spaetzle are produced by preparing fresh dough from flour, water, salt and eggs, forming it into hazelnut-sized pieces, and cooking these pieces in boiling water for 12–15 min. Several small family outbreaks due to *Salmonella* reported in Western Austria within the past 5 years were also suspected of being due to an insufficient boiling time for this traditional food item (data not published).

Analysis of the food-specific attack rates by day as described by Kuo et al. [13], which considers the time sequence of exposure and disease status, confirmed the spaetzle (at dinner on August 25) at borderline significance and the hamburger (at lunch on August 26) as risk-associated items and additionally identified the potato salad served for dinner on August 26 as a likely infection source for soccer club A, the only club for which the day and time of food exposure were known. The pattern of the outbreak curve for the club A cases, which indicated that the outbreak sources were active on August 25 and 26, agrees with the findings of the day-wise food-specific analyses. Based on these findings, it was assumed that eggs were the most likely vehicle of the outbreak strain. In Austria, hamburger is traditionally prepared with eggs. The potato salad served at dinner could have easily been cross-contaminated during preparation, including during the peeling and cutting of the boiled potatoes, in parallel with the preparation of the egg-containing hamburgers for lunch on the same day. During the storage of the potato salad between lunch and dinner, bacteria would have had enough time to multiply. Inadequate hand hygiene practices between preparing the hamburgers and the potato salad could easily have enabled cross-contamination with salmonella from the *S. enteritidis* PT4-positive eggs. In Austria, potato salad is a well-documented source of *S. enteritidis* infection [14,15].

In 2010, the sole egg producer supplying hotel X with table eggs was a local laying hen holding that had been epidemiologically and microbiologically related to a previous outbreak with four cases of *S. enteritidis* PT4 in the same province. Two cases with disease onset in May and June had lunch in a local restaurant provided with table eggs from this laying hen holding, whereas the other two cases with disease onset in July consumed “meals on wheels” (an aid program delivering meals to individuals at home who are unable to prepare their



**Figure 2** Cluster analysis of *Xba*I-PFGE fragment patterns of 17 outbreak-related *Salmonella enteritidis* PT 4 isolates including human isolates from the 4 cases of the previously flock A-related PT4 outbreak (prev\_outbreak cases I–IV: lanes 2, 3, 6, 7), human isolates from the 10 cases of the soccer camp outbreak (soccer\_camp outbreak cases I–X: lanes 9–18), non-human isolates from two dust samples (dust samples I, II: lanes 4, 5) and a pooled egg sample (egg sample: lane 8). One major cluster including 13 of the 14 human isolates and all of the 3 non-human isolates was identified. The isolate from soccer camp\_outbreak IV (lane 12) was considered to be closely related.

own food) prepared in the hotel X kitchen using table eggs from the same laying hen holding. Both flocks of the laying hen holding were vaccinated, but vaccination of laying hen flocks cannot prevent a low degree of bacterial shedding [16,17]. Following the occurrence of the first two cases of this preceding PT4 outbreak, flock A and flock B of the laying hen holding were investigated by local public health authorities. Flock A tested positive for *S. enteritidis* PT4 in two dust samples and in two of three boot swab samples pooled from five paired boot swabs (unpublished results), whereas flock B tested negative for *S. enteritidis* PT4 in all of the samples.

At that time, the inspection of the hotel X kitchen had already revealed unclean conditions in the kitchen and storage room, inadequate hand hygiene practices and a lack of a hazard analysis and critical control points concept. Eggs from flock A were prohibited from sale as table eggs as of July 14, 2010, when a marketing ban was issued. After eggs sampled on July 21 also tested positive for *S. enteritidis* PT4, flock A was culled on August 9, 2010. Even though the marketing ban for table eggs from flock A was issued on July 14, five weeks prior to the onset of the soccer camp outbreak associated with hotel X on August 20, flock A still had to be suspected as the reservoir for the soccer camp outbreak. Specifically, the possibility was considered by the public health authorities that eggs derived from flock A were falsely declared as table eggs

and sold among eggs from the *Salmonella*-negative flock B until flock A was culled on August 9. As a result, the public health authorities filed charges. The prosecution eventually dropped the case without further investigation.

Driven by the economic consequences of egg restrictions and the requirement to heat-treat eggs from *Salmonella*-positive flocks, economic pressures or greed-for-gain can mislead egg producers into counteracting measures of risk management. In July 2010, an Austrian egg producer admitted to having distributed a total of 1.3 million eggs originating from Hungary and Poland that were falsely declared as Austrian eggs [18,19]. In October 2009, another Austrian egg farm was linked to a cluster of 15 cases of *S. enteritidis* although a marketing ban had been imposed on the eggs from that farm since the end of September; eggs intended for industrial use only were deliberately mixed with table eggs during packaging and distribution [20]. These two episodes underscore the fact that the illegal distribution of eggs, i.e., the false declaration of eggs determined for industrial use as table eggs, does occur.

Zoonoses Directive 2003/99/EC specifies that competent authorities must investigate suspected foodborne outbreaks. According to the European Commission, the findings of a thorough investigation of zoonotic foodborne outbreaks provide the opportunity to improve control and prevention measures for foodborne diseases [21]. The

outbreak described in this report underscores the potential of proper outbreak investigation to identify the pitfalls of regulatory responses in risk management.

## Conflict of interest statement

**Funding:** No funding sources.

**Competing interests:** None declared.

**Ethical approval:** Not required.

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